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RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of two Drugs Nitazoxanide, Ofloxacin and its Pharmaceutical Dosage Forms

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ABSTRACT

Establishment of a single analytical method for estimation of individual drug from a multi-drug composition is a very challenging task. A rapid, simple and precise HPLC method was developed for the separation and estimation of two drugs Nitazoxanide and Ofloxacin from bulk drug mix and pharmaceutical dosage forms. The estimation was carried out using Luna C18 (250mm x 4.6mm, 5µm) column; mobile phase consisting of Acetonitrile and buffer at pH 4; the flow rate of 1.5ml/min and ultraviolet detection at 280 nm. Both drugs were properly resolved having run time of 3.7 min and 1.5 min for Nitazoxanide and Ofloxacin, respectively. The method was validated as a final verification of method development with respect to Precision, Linearity, Accuracy, Ruggedness and Robustness. The validated method was successfully applied to the commercially available pharmaceutical dosage form, yielding very good and reproducible result.

Keywords: Nitazoxanide, Ofloxacin HPLC, LC Method development and validation.

I.INTRODUCTION

Nitazoxanide is used to treat diarrhea in children and adults caused by the protozoa Cryptosporidium or Giardia. Nitazoxanide is in a class of medication called antiprotozoal agent. It works by stopping the growth of protozoa that causes diarrhea. It is chemically 2-(acetolyloxy)-*N*-(5-nitro- 2-thiazolyl) benzamide and its structure is as shown in Figure 1. Ofloxacin is a class of antibiotic called fluoroquinolones that stops bacterial multiplication by inhibiting the reproduction of the genetic material (DNA). It is chemically (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-zatricyclo[7.3.1.0] trideca-5(13),6,8,11-tetraene-11-carboxylic acid and its structure is as shown in Figure 1.

There are couple of methods[7-10] available for the simultaneous estimation of both the drugs but they have their own limitations. In this article, a very fast and user-friendly method for the simultaneous estimation of Nitazoxanide and Ofloxacin using reverse phase- HPLC method in bulk drug mix and pharmaceutical dosage forms.

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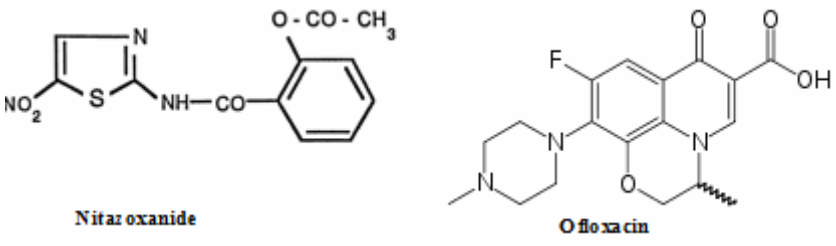
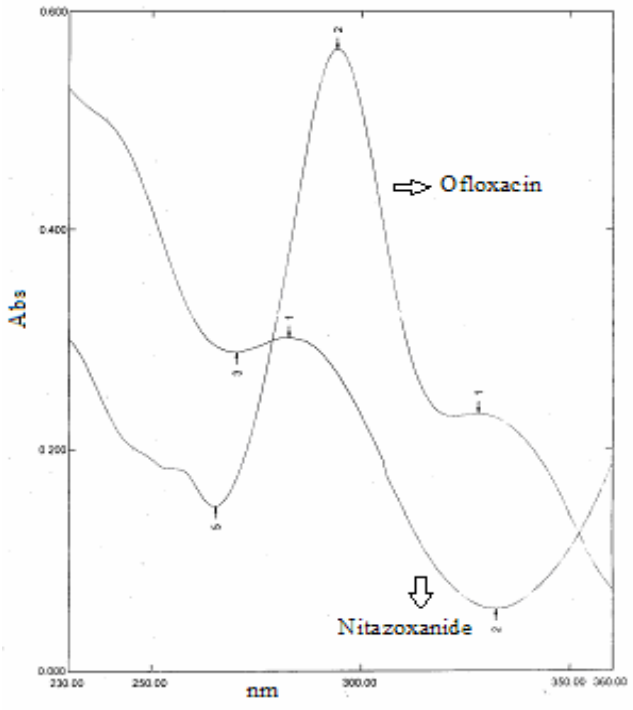


Figure 1: Structure of Nitazoxanide and Ofloxacin

II.EXPERIMENTAL SECTION

2.1. METHOD DEVELOPMENT

Both the drugs were scanned by UV, individually, in a wavelength range of 200-400 nm and maxima for each drug was measured. The maxima for Nitazoxanide was found to be 284.60nm, 330.60nm and 269.60nm whereas, for Ofloxacin maxima were found at 328.00nm, 294.20nm and 226.40nm. The corresponding UV spectrum graphs of the drugs Nitazoxanide and Ofloxacin are as shown in Figure 2. To optimize the UV maxima, various HPLC experiments were performed at different wavelengths starting from 240nm to 290nm.



	Nitazoxanide	Ofloxacin
1	284.60nm	328.00nm
2	330.60nm	294.20nm
3	269.60nm	226.40nm

Figure 2: UV Spectrum graph

It was observed that both peaks were resolving at all six wave numbers namely, 240nm, 250nm, 260nm, 270nm, 280nm and 290nm but experiment ofHPLC run at 280 nm has been found to be better with respect to resolution of the peaks and balanced area acquisition of both drugs. Hence, wavelengthof280 nm was finalized for the data acquisition in HPLC for the simultaneous estimation of both the drugs.

2.2. ANALYTICAL METHOD VALIDATION

2.2.1. SPECIFICITY OF THEMETHOD

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. This parameter was performed to know the Retention Time of each drug in a mixture and in the sample to understand if any drug-drug interaction or drug-excipient interaction is present.

2.2.2. SYSTEM SUITABILITY

System suitability test is used to verify if the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The limits for system suitability were set for Theoretical plates, Resolution, Asymmetry.

2.2.3. ACCURACY

To determine the accuracy in sample preparation method of standard additions was made for measuring the recovery of the drugs. To the known standard solution concentrations of the drug (50%, 75%, 100%, 125%, and 150%) was added. Five different solutions were prepared as mentioned in section 2.7. The accuracy was expressed as the percentage of the analytes recovery.

2.2.4. METHODPRECISION

It is very important that the method developed should be precise. Six replicates of the sample prepared from the commercial tablets were injected and Assay was calculated to measure the repeatability of retention times and peak area of standard and sample.

2.2.5. RUGGEDNESS

To test the ruggedness of the method, the analysis was done on different days and different chemists to check for any changes in the chromatograph. The percentage RSD for the retention time and area was calculated.

2.2.6. PERFORMANCE OF THE METHOD ON COMMERCIAL SAMPLES

The method is said to be effective if it can be applied for the analysis of commercial tablets. For this purpose, performance test of the method has been conducted on two market samples NITA-O, manufactured by Alembic limited, Batch No 10321004 and Zenflox NT manufactured by Windlas Biotech Limited, Batch No ZNX35.

III.RESULTS AND DISCUSSION

After several experiments above method has been optimized.the current method has been developed and it is very fast and encouraging. The developed method was validated with a holistic approach according to ICH

guidelines and details of findings are expressed in following lines.

3.1. SPECIFICITY OF THEMETHOD

The Retention times of the standard drugs individually were measured and it was found to be 3.750 min and 1.533min for Nitazoxanide and Ofloxacin, respectively. The drugs were mixed and injected for taking the chromatogram. Both drugs were resolved very nicely in the mixture. Retention time of both drugs in Standard mix was found to be 3.760 min and 1.542 min for Nitazoxanide and Ofloxacin, respectively. This indicates there is no drug- drug interaction.

3.2. SYSTEM SUITABILILTY:

Five injections of the standard mix and two injections of the sample were injected for this purpose. The Resolution, Areas, Retention time,Theoretical plates values and peak Asymmetry were calculated for standard and sample solutions. Results obtained are given in following Table1.

Table 1: System suitability Results

Standard		Average	SD	% RSD
Nitazoxanide	Retention Time	3.763	0.004	0.13
	Area	8737421	22507.9	0.26
	Resolution	14.874	0.08	0.56
	Theoretical Plates	10333	-	-
Asymmetry		1.15	-	-
Ofloxacin	Retention Time	1.542	0	0
	Area	11064903	19876.52	0.18
	Resolution	0	0	0
	Theoretical Plates	1676.006	-	-
Asymmetry		1.482	-	-
Dosage form				
Nitazoxanide	Retention Time	3.758	0	0
	Area	8664919	15152.59	0.17
	Resolution	14.75	0.18	1.24
	Theoretical Plates	10049.16	-	-
Asymmetry		1.095	-	-
Ofloxacin	Retention Time	1.542	0	0
	Area	11057507	16247.9	0.14
	Resolution	--	--	--
	Theoretical Plates	1676.93	-	-

Asymmetry	1.495	-	-

3.3. LINEARITY:

The correlation coefficient (r) obtained was calculated and it was found to be greater than 0.99 for Nitazoxanide and Ofloxacin, which is well within the acceptance criteria. The results are shown in Table 2. The concentrationwas found to be proportional to the area and the response of the detector was determined to be linear over the range of 0.2 to 0.6mg/ml for Ofloxacin and0.5 to 1.5mg/ml for Nitazoxanide as shown in the Figure 3.

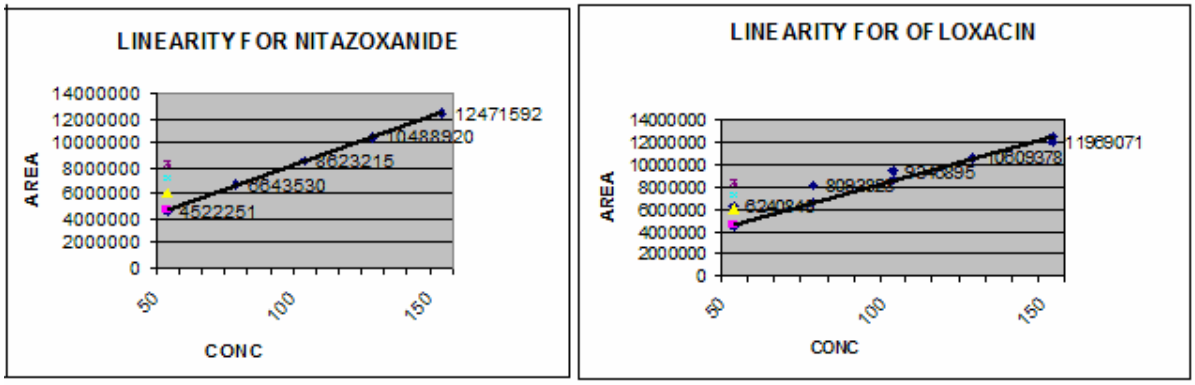


Figure 3: Graphs Showing Linearity of the drugs

Table 2: Linearity results

	Linearity Range	Correlation Coefficient
Nitazoxanide	0.5-1.5mg/ml	0.9998
Ofloxacin	0.2-0.6mg/ml	0.9969

3.4. METHOD PRECISION

The percentage RSD values for Area and Retention Time in precision study were calculated. The results asshown in Table 3 indicate that the method developed is precise.

Table 3: Method Precision results

	Retention Time		**Area**	
	Nitazoxanide	**Ofloxacin**	**Nitazoxanide**	**Ofloxacin**
1	3.695	1.501	8563271	10587458

2	3.697	1.498	8564315	10596371
3	3.698	1.5	8607243	10613872
4	3.699	1.512	8617425	10638541
5	3.698	1.511	8653482	10625874
Average	3.697	1.504	8601147	10612423
%RSD	0.44	0.44	0.44	0.2

IV.CONCLUSION

A unique, user friendly, rapid and reproducible HPLC Method for simultaneous estimation of Nitazoxanide and Ofloxacin in Pharmaceutical dosages forms has been developed and validated as per ICH Guidelines. Therefore, this method can be used by the industries and academic institutions for their combination drug estimation, which is fast as well as safe.

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